Taiwanese Journal of Obstetrics & Gynecology 56 (2017) 456–462

Contents lists available at ScienceDirect

Taiwanese Journal of Obstetrics & Gynecology

journal homepage: www.tjog-online.com

Original Article

The effect of oxytocin and Kisspeptin-10 in ovary and uterus of ischemia-reperfusion injured rats

M. Aslan [a, 1], G. Erkanli Senturk [b, *, 1], H. Akkaya [c], S. Sahin [d], B. Yılmaz [e]

[a] Bahcesehir University, School of Medicine, Istanbul, Turkey
[b] Bahcesehir University, School of Medicine, Department of Histology and Embryology, Goztepe, Istanbul, Turkey
[c] Yeditepe University, School of Medicine, Experimental Research Center, Kayisdagi, Istanbul, Turkey
[d] Istanbul Medeniyet University, Goztepe Research and Education Hospital, Department of Obstetrics and Gynecology, Goztepe, Istanbul, Turkey
[e] Yeditepe University, School of Medicine, Department of Physiology, Kayisdagi, Istanbul, Turkey

ARTICLE INFO

Article history:
Accepted 20 December 2016

Keywords:
Kisspeptin
Oxytocin
Uterus
Ovary
Ischemia-reperfusion

ABSTRACT

Objective: Ischemia/reperfusion (I/R) injuries result in damage to endothelial and parenchymal cells. Oxytocin (OXY) stimulates uterine contraction during parturition and myoepithelial cells during suckling. OXY has been used as a protective antioxidant. Kisspeptin plays a key role in the central control of reproductive functions and onset of puberty. Recent studies show that these reproductive hormones have protective potential as antioxidant. The aim of this study is to investigate the potential protective effects of Kisspeptin and OXY as antioxidants on I/R injured ovary and uterus of female rats.

Materials and methods: Rats were separated into five groups. Group 1, is control group; Group 2, rats were subjected to ischemia followed by reperfusion. Group 3, OXY administration 30 min prior to I/R applied rats; Group 4, Kisspeptin administration 30 min prior to I/R applied rats; Group 5, OXY and Kisspeptin administration 30 min prior to I/R. Ovary and uterus were removed for histopathological and biochemical observations. Malondialdehyde, glutathione levels, and superoxide dismutase activities were analyzed in order to observe antioxidant potential of OXY and Kisspeptin. Hematoxylin and Eosin staining was applied for histopathologic scoring.

Results: Stromal and granulosa cells in ovary, endometrial cells in uterus were damaged in I/R group. The cellular damage of ovary and uterus were reduced in OXY and Kisspeptin administered I/R group when compared to only Kisspeptin injected I/R group and I/R group. There is no significant difference between OXY and OXY + Kisspeptin injected I/R groups. MDA levels were decreased in Kisspeptin and/or Oxytocin applied I/R group compared to I/R group. SOD activity and GSH levels were increased in Kisspeptin and/or OXY applied I/R group compared to I/R group.

Conclusions: The present results suggest that exogenous application of oxytocin and kisspeptin can have antioxidant effects on the uterus and ovary.

Introduction

Ovarian torsion with a prevalence of 2.7% is a gynecological emergency. Ovarian torsion causes ischemia in the tissue and the blood flow can be restored by surgical intervention. The diagnosis and the treatment may be delayed because of the non-specific symptoms and clinical findings of this pathological condition. Detorsion, laparoscopy or laparotomy can be performed for twisted adnexa. Detorsion causes ischemia/reperfusion injury while providing circulation to ovary. Reactive oxygen species (ROS) released during reperfusion worsens acute ischemic injury [1].

Overproduction of ROS, such as superoxide anion, nitric oxide (NO), hydrogen peroxide, hydroxyl radical and free radicals causes some pathological states, although normal amounts of them are vital for normal physiology. The cells are protected against ROS damage by various ways such as scavenging enzyme systems including Catalase which converts hydrogen peroxide into hydrogen oxide (water) and superoxide dismutase (SOD). SOD catalyzes the

* Corresponding author. Present adress: Istanbul University, Cerrahpasa Medical Faculty, Department of Histology and Embryology, Istanbul, Turkey.
E-mail addresses: gozdeerkanli@yahoo.com, gozde.erkanlisenturk@istanbul.edu.tr (G. Erkanli Senturk).
[1] These authors contributed equally to the study.

http://dx.doi.org/10.1016/j.tjog.2016.12.018

partitioning of the superoxide (O^{2-}) radical into either ordinary molecular oxygen (O^2) or hydrogen peroxide (H^2O^2). Superoxide is produced as a by-product of oxygen metabolism. It needs to be regulated; otherwise it causes many types of cell damage. Lipid peroxidation which plays a role in decreased binding of hypothalamic hormones to their receptors causes a decline in antioxidant systems [2].

Oxytocin which is a nonapeptide produced in paraventricular and supraoptical nuclei of hypothalamus have central and peripheral effects. It plays a role in uterine contraction, milk ejection reflex, cardiovascular and hydroelectrolytic regulations and modulation of release of adenohypophyseal hormones as well as behaviors such as maternal, sexual and social. Many tissues such as heart, thymus and adipocytes contain OXY receptors. Recent studies show that OXY has a role in wound healing and modulation of immune and inflammatory processes by acting as acute phase reactants and interleukins. OXY may also activate the growth factors and anti-inflammatory mechanisms. Therefore, it helps the ischemic skin flaps to survive [1].

Kisspeptin (metastin) is a 145 amino acid protein which has a role in controlling reproductive functions and puberty with its G protein coupled membrane receptors (GPR 54). Studies reveal that kisspeptin induces the hypothalamic pituitary gonadal axis and adjusts an antioxidant enzyme expression against oxidative damage [3].

The goal of this study was to evaluate the protective effect of exogenous oxytocin and kisspeptin as antioxidants, together or alone, against ischemia/reperfusion injury in ovary and uterus.

Material-method

Animals

The study was performed on the 24 adult Wistar-Albino rats with body weight of 150–200 g in accordance with institutional guidelines. Animals were placed in a quiet and temperature (22 ± 2 °C) and humidity (60 ± 5%)-controlled room in which a 12/12 h light/dark cycle was maintained. All experiments, scheduled between 09:00 and 17:00 h, were performed in accordance with the guidelines for animal research and were approved by the Yeditepe University Ethical Committee of Animal Care as approved number 434, Istanbul, Turkey.

Experimental procedure

The rats were anesthetized with a combination of 50 mg/kg ketamine hydrochloride and 7 mg/kg xylazine hydrochloride, intraperitoneally. The depth of the anesthesia was kept at a level to preserve spontaneous respiration while providing necessary analgesia. No other venous cannula was inserted and all animals spontaneously breathed the room air during surgical procedures. All animals were anticoagulated with intravenous heparin 10 min before the ischemia to prevent thrombosis in the occluded artery.

The abdominal aorta, just above the bifurcation point, was exposed except control group. In the control group, the abdominal aorta was not occluded. Blood-flow occlusion was confirmed with the visual assessment of color changes of the sole of the foot, by palpation of bifurcation point of abdominal aorta pulse. I/R procedure were done according to our previous data (1). Oxytocin (0.5 μg/kg was dissolved in phosphate-buffered saline (PBS), Sigma–Aldrich Co., St Louis, MO, USA) was injected intraperitoneally (i.p.) 30 min before the ischemia in the I/R + OXY group (n:6), Kisspeptin (0.5 μg/kg was dissolved in phosphate-buffered saline (PBS), Sigma–Aldrich Co., St Louis, MO, USA) was injected intraperitoneally (i.p.) 30 min before the ischemia in the I/R + Kiss group (n:6) whereas the vehicle solution of an equal volume was injected 30 min before the ischemia in the I/R group (n:6). The ovary and uterus was rendered ischemic for 90 min and reperfusion was achieved by releasing the clamp and was confirmed by restoration of the pulsatile blood flow in the aorta, and disappearance of color change of the sole. Animals in I/R group (n:6) were subjected to 90 min of abdominal aorta occlusion followed by 90 min reperfusion. In oxytocin pretreated I/R group (I/R + OXY) (n:6), oxytocin were given i.p. 30 min before ischemia. In kisspeptin pretreated I/R group (I/R + Kiss) (n:6), kisspeptin were given i.p. 30 min before ischemia. In oxytocin and kisspeptin pretreated I/R group (I/R + OXY + Kiss) (n:6), oxytocin and kisspeptin were given i.p. 30 min before ischemia. At the end of the reperfusion period of 90 min, right ovary and uterus tissue samples were taken from all groups to assess histopathology and left ovary and uterus tissue samples were taken for freeze clamp biopsies for biochemical analysis from all groups. All animals survived until the end of the reperfusion period.

Histopathologic examination

Samples from the ovary and uterus were incubated in 10% formalin solution and routinely processed afterwards, embedded in paraffin blocks. 5 μm ovary and uterus tissue sections were stained with hematoxylin and eosin (H&E) for histopathological scoring. Every fifth section for each block was evaluated from all animals for both uterus and ovary by a blind observer. The stained sections were examined by Leica BM500 (German) photomicroscope for light microscopical examination. All sections were scored according to below scoring system.

Scoring of histopathological damage was performed for uterus: vasocongestion, infiltration of inflammatory cells, epithelial desquamation [4]. And for ovary: degeneration of follicles in the cortical area (cellular dispersion and degeneration of follicular cells), vascular congestion, haemorrhaging, oedema, infiltration by inflammatory cells [11]. Each criterion was evaluated as according to normal (0), mild (1), moderate (2), severe (3). Maximum score was 9 for uterus and 15 for ovary. All results were calculated as mean ± SD statistically.

Biochemical examination

Malondialdehyde (MDA) analysis

MDA determination is used for lipid peroxidation. The lipid peroxidation level in the ovary and uterus tissue was measured according to the concentration of thiobarbituric acid reactive substances [21]. Briefly, one volume of the test sample and two volumes of stock reagent (trichloroacetic acid and thiobarbituric acid) were mixed in a centrifuge tube. The solution was heated in boiling water for 20 min. After cooling, the precipitate was removed by centrifugation at 2500 rpm for 5 min, and then absorbance of the supernatant was read at 532 nm against a blank containing all reagents except test sample on a spectrophotometer. The thiobarbituric acid reactive substance level was expressed as nanomoles per gram tissue.

Total glutathione (GSH) analysis

GSH plays role as a co-substrate in the metabolism of xenobiotics and it is also a co-factor for several metabolic enzymes and is involved in intracellular transport, functions as an antioxidant and radioprotectant and facilitates protein folding and degradation. GSH (μmol/mL) was determined by a spectrophotometric method based on the use of Ellman's reagent [5]. The samples were precipitated by a solution containing metaphosphoric acid, disodium EDTA, and then centrifuged at 3000 rpm for 30 min. The reaction mixture contained 0.25 mL of supernatant, 1 mL of Na_2

HPO_4, and 0.125 mL of 5.5-dithio-bis-2-nitrobenzoicacid. The solution was kept at room temperature for 5 min, and then read at 412 nm on the spectrophotometer.

Superoxide dismutase (SOD) analysis

SOD catalyzes superoxide anion to molecular oxygen and hydrogen peroxide, since it provide the essential part of cellular antioxidant defense mechanism. SOD (U/mg protein) activity was determined as described by Sun et al. [6]. Briefly, one volume of test sample and one volume of chloroform and ethanol mixture (3/5 v/v) were mixed in a centrifuge tube. The precipitate was removed by centrifugation at 3500 rpm for 45 min. The assay solution containing sodium carbonate buffer (400 mM), 0.3 mM xanthine, 150 μmol/L nitroblue tetrazolium (NBT), 0.6 mmol/L Na_2EDTA, 1 g/L bovine serum albumin, 167 U/L xanthine oxidase, and sample were mixed in a cuvette. The activity was measured using xanthine and xanthine oxidase to generate superoxide radicals, which react with NBT. One SOD unit was defined as the amount of protein that inhibits the rate of NBT reduction by 50%; SOD activities were expressed as U/mg protein. The protein content was determined according to the method of Lowry et al. using bovine serum albumin [7].

Biostatistical analysis

Graph Pad Prism 3.0 (Graph Pad Software, San Diego, CA, USA) were used for statistical analysis of all data. All data are calculated as mean ± S.D. Using an analysis of variance (ANOVA) followed by Tukey's multiple comparison tests were carried out for the results of MDA, GSH, SOD and histopathological damage scores.

Results

Histopathological evaluation

Normal endometrial structures were observed in the uterine control group (Fig. 1A). However, there was epithelial degeneration,

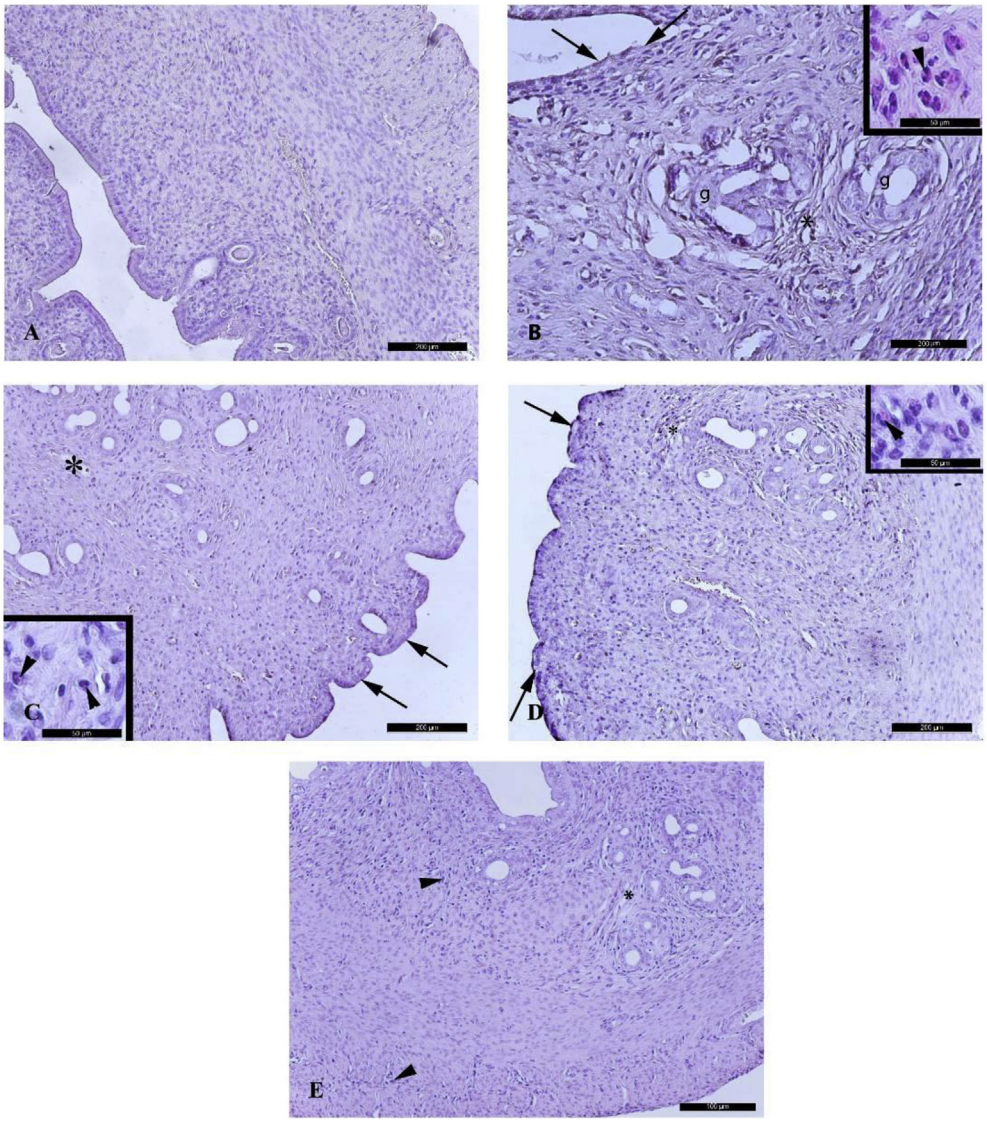

Fig. 1. (A) Control group with normal endometrial structures; **B)** epithelial degeneration (→), interstitial oedema (*), glandular cell degeneration (g) and inflammatory cell infiltration (, insert) in ischemia/reperfusion group; **C)** moderate degeneration of epithelium (→) and oedema (*) with few inflammatory cells (►, insert) in OXY applied ischemia/reperfusion group; **D)** moderate epithelial degeneration (→), interstitial oedema (*), inflammatory cell infiltration (►, insert) in Kisspeptin applied ischemia/reperfusion group; **E)** Almost normal morphology of uterine tissue with few inflammatory cell (→) and decreased oedema (*) in OXY and Kisspeptin applied ischemia/reperfusion group.

intertial oedema, glandular cell degeneration and inflammatory cell infiltration in the uterine Ischemia/Reperfusion group (Fig. 1B). Moderate degeneration of epithelium and oedema with few inflammatory cells in OXY + IR group were observed (Fig. 1C). There was moderate epithelial degeneration, interstitial oedema, inflammatory cell infiltration in KISS + IR group (Fig. 1D). Almost normal morphology of uterine tissue with few inflammatory cell and decreased oedema were observed in OXY + KISS + I/R group (Fig. 1E). The histological damage scores of uterus in the I/R ($p < 0.001$) and OXY + I/R ($p < 0.001$), KISS + I/R ($p < 0.001$) and OXY + KISS + I/R ($P < 0.05$) groups were significantly higher than those of the control group (Fig. 3). In addition, histological damage scores of uterus in the OXY + I/R ($p < 0.01$), KISS + I/R ($p < 0.05$) and OXY + KISS + I/R ($p < 0.001$) group were significantly lower than those in the I/R group (Fig. 3).

Normal morphology of tertiary follicles with oocyte was observed in ovarian tissue of control group (Fig. 2A). Degenerated primary follicle, vascular congestion, oedema were seen in I/R group (Fig. 2B). Almost normal morphology of early tertiary follicles with oocyte was demonstrated in OXY + I/R group (Fig. 2C). Degenerated primary follicle, vascular congestion, oedema were observed in KISS + I/R group (Fig. 2D). Almost normal morphology of primary follicle with oocyte was seen in ovary of OXY + KISS + I/R group (Fig. 2E). The histological damage scores of ovary in the I/R ($p < 0.001$) and KISS + I/R ($p < 0.01$) groups were significantly higher than those of the control group (Fig. 4). In addition, histological damage scores of uterus in the OXY + I/R ($p < 0.05$) and OXY + KISS + I/R ($p < 0.001$) group were significantly lower than those in the I/R group. Moreover, OXY + KISS + I/R group was significantly different than KISS + I/R ($p < 0.01$) group (Fig. 4).

Biochemical analysis of GSH, MDA and SOD levels

For uterus, GSH level has been reduced significantly in the I/R group compared to the control group (6.13 ± 1.6 μmol/g versus 8.18 ± 4.1 μmol/g; $P < 0.05$). However, this marked reduction in GSH levels has been reversed in OXY + I/R group (8.90 ± 3.4 μmol/g; $P < 0.05$) and OXY + KISS + I/R group (7.97 ± 4.5, $p < 0.05$) as close

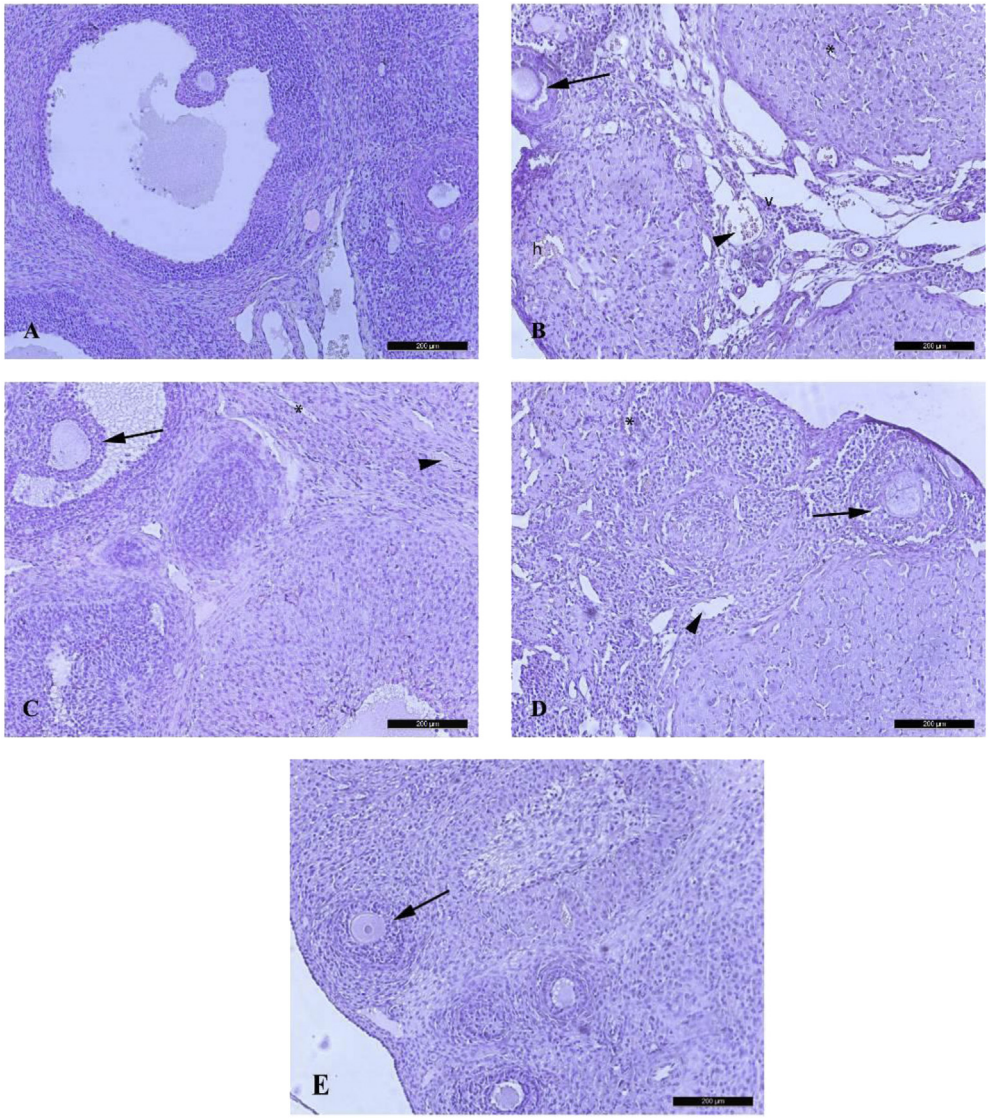

Fig. 2. **(A)** Normal morphology of tertiary follicles with oocyte in control group; **B)** Degenerated primary follicle (→), vascular congestion (►), oedema (*) in ischemia/reperfusion group. **C)** Almost normal morphology of early tertiary follicles with oocyte in OXY applied ischemia/reperfusion group; **D)** Degenerated primary follicle (→), vascular congestion (►), oedema (*) in Kisspeptin applied ischemia/reperfusion group. **E)** Almost normal morphology of primary follicle (→) with oocyte in OXY + KISS + I/R group.

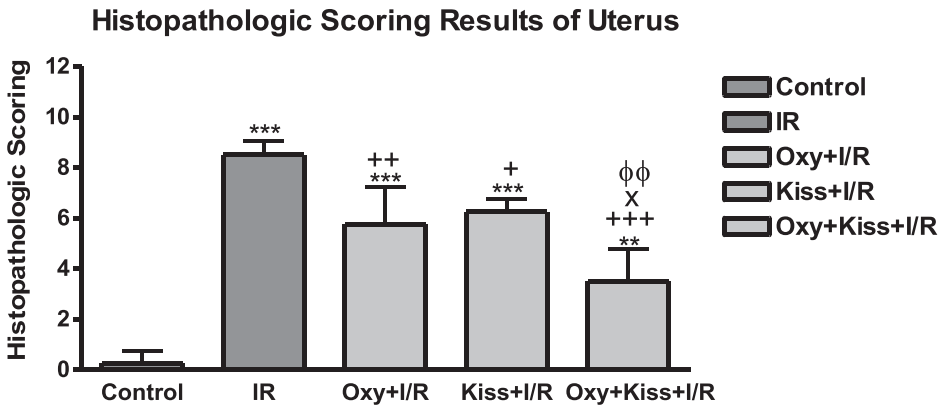

Fig. 3. Histopathologic scoring results of uterus. ***; $p < 0.001$, **; $p < 0.01$ compared with control group. +++; $p < 0.001$, ++; $p < 0.01$, +; $p < 0.05$ compared with ischemia/reperfusion group. X; $p < 0.05$ compared with OXY applied ischemia/reperfusion group. ϕϕ; $p < 0.01$ compared with Kisspeptin applied ischemia/reperfusion group.

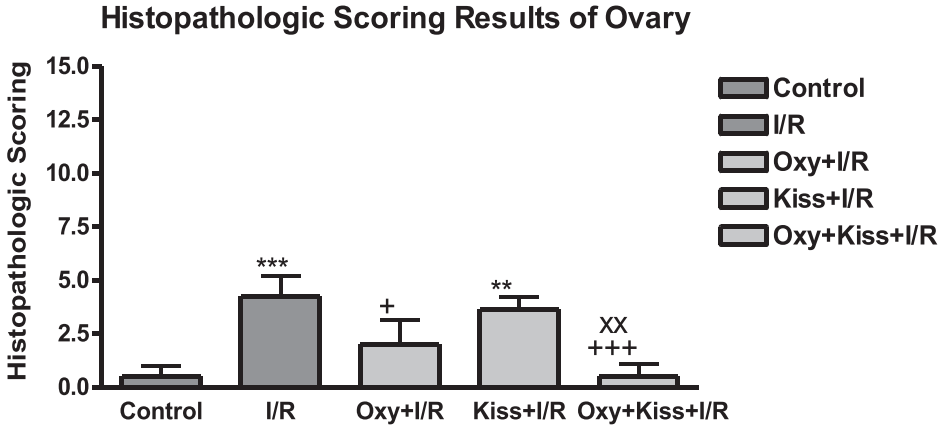

Fig. 4. Histopathologic scoring results of ovary. ***; $p < 0.001$, **; $p < 0.01$ compared with control group. +++; $p < 0.001$, +; $p < 0.05$ compared with ischemia/reperfusion group. XX; $p < 0.01$ compared with OXY applied ischemia/reperfusion group.

to GSH level of the control and higher than that in the I/R group. A significant increase of lipid peroxidation was determined based on MDA analysis in the I/R uterus. The mean MDA level in the uterus tissues of the control group was determined as 2.89 ± 1.2 nmol/g whereas it was 4.86 ± 1.3 nmol/g in the I/R group ($P < 0.05$). The MDA level was 2,68 ± 0,5 nmol/g in the OXY + I/R group ($P < 0.05$) and 2.72 ± 0.4 nmol/g in the OXY + KISS + I/R group ($P < 0.05$), which was significantly lower than that in the I/R group and it approached to the value of the control group (Fig. 4). SOD level of the uterus tissue has been reduced significantly in the I/R group compared to the control group (2.4 ± 0.7 U/mg versus 4.12 ± 0.8 U/mg; $P < 0.05$). SOD levels were increased in oxytocin and/or kisspeptin applied I/R groups ($p < 0.05$) when compared to I/R group (Table 1).

For ovary, GSH level has been reduced significantly in the I/R group compared to the control group (6.22 ± 1.1 μmol/g versus 10.31 ± 1.7 μmol/g; $P < 0.05$). However, this marked reduction in GSH levels has been reversed in OXY + I/R group (9.46 ± 1.1 μmol/g; $P < 0.05$) and OXY + KISS + I/R group (9.01 ± 1.1; $p < 0.05$) as close to GSH level of the control and higher than that in the I/R group. A significant increase of lipid peroxidation was determined based on MDA analysis in the I/R ovary. The mean MDA level in the ovary tissues of the control group was determined as 2.26 ± 0.6 nmol/g whereas it was 3.24 ± 0.5 nmol/g in the I/R group ($P < 0.05$). The MDA level was 1.19 ± 0.07 nmol/g in the OXY + I/R group ($P < 0.05$) and 2.34 ± 0.2 nmol/g in the OXY + KISS + I/R group ($P < 0.05$), which was significantly lower than that in the I/R group and it approached to the value of the control group. SOD level of the ovary has been reduced significantly in the I/R group compared to the control group (3.81 ± 1.5 U/mg versus 5.35 ± 1.8 U/mg; $P < 0.05$). SOD levels were increased in oxytocin and/or kisspeptin applied I/R groups ($p < 0.05$) when compared to no applied I/R group (Table 1).

Table 1
Biochemical results of superoxide dismutase (SOD), total glutathione (GSH), and malondialdehyde (MDA) in uterus and ovarian tissue.

Uterus	SOD (U/mg protein)	GSH (μmol/gr)	MDA (nmol/gr)
Control	4.12 ± 0.8	8.18 ± 4.1	2.89 ± 1.2
I/R	2.4 ± 0.7*	6.13 ± 1.6*	4.86 ± 1.3*
OXY + I/R	5.12 ± 1.6+	8.90 ± 1.4+	2.68 ± 0.5+
Kiss + I/R	4.55 ± 1.60+	8.34 ± 1.4+	3.38 ± 0.1+
OXY + Kiss + I/R	5.9 ± 0.9+	7.97 ± 0.5+	2.72 ± 0.4+
Ovary	**SOD (U/mg protein)**	**GSH (μmol/gr)**	**MDA (nmol/gr)**
Control	5.35 ± 1.8	10.31 ± 1.7	2.26 ± 0.6
I/R	3.81 ±v1.5*	6.22 ± 1.1*	3.24 ± 0.5*
OXY + I/R	5.44 ±v1.8+	9.46 ± 1.1+	1.19 ± 0.07+
Kiss + I/R	4.52 ± 0.60+	8.89 ± 1.4+	1.62 ± 1.1+
OXY + Kiss + I/R	5.33 ± 2.8+	9.01 ± 1.1+	2.34 ± 0.2+

*; $p < 0.05$ compared with control group. +; $p < 0.05$ compared with ischemia/reperfusion group.

Discussion

We have evaluated the Oxytocin and Kisspeptin-10 alone and together effect on oxidative stress in ovarian and uterus tissue of ischemia-reperfused rats. Additionally, we observed that kisspeptin-10 and/or Oxytocin treatment protects ovarian and uterus from I/R injury. Oxytocin and/or Kisspeptin-10 treatment decreased I/R induced histopathologic damage, which was related with the diminished MDA level and increased SOD and GSH levels.

Anaerobic glycolysis occurs in cells and lactate is produced consequently when the arterial blood supply diminishes. Additionally, ATP-dependent ion transport is disturbed on the cell membrane and the mechanisms of cell death occurs [8,9]. Ischemia after reperfusion may also cause tissue damage due to ROS and many mediators produced with neutrophil accumulation [10]. ROS triggers cell damage by free radical production. These radicals react with the lipids on the cell membrane, and lipid peroxidation causes cell damage and acute inflammatory response. Many pharmaceutical agents with antioxidant and/or anti-inflammatory effects have been studied for protective effects against ovarian I/R injury in animal models. Akdemir et al. reported that oxytocin had protective effects against ovarian I/R injury in rats [11]. Oxytocin ameliorates histopathological parameters such as vascular congestion, edema and so on, and decrease of oxidative stress parameters in ovarian tissue [11]. Malondialdehyde is one of the end products of lipid peroxidation, and it increases in oxidative stress [12]. The common oxidative damage indicators involved in the first defence mechanism are antioxidants such as superoxide dismutase (SOD), catalase and GSH. SOD and catalase enzymes catalyze the conversion of ROS into less reactive species. GSH is the most important endogenous antioxidant [13]. As our present study, previous studies showed that both tissue and serum MDA increased, SOD and GSH decreased as a result of I/R injury. Moreover, some applied drugs decreased serum and/or tissue MDA and increased SOD and GSH in rats with ovarian I/R injury [14].

In our study, MDA, SOD and GSH were used as an antioxidant marker. And we found out that oxytocin and kisspeptin increased SOD and GSH levels and decreased MDA level, in parallel with histopathologic tissue damage recovery in ovary and uterus.

Oxidants can react with cellular molecules and structural components and modify their properties. Such interactions can change the "Ca^{+2} code" and modify essential pathways [15]. Calcium is a second messenger and can be used as a signal molecule to respond to oxidant stimuli. It has recently been reported that kisspeptin-10 regulates gonadotropin-releasing hormone secretion in GT1-7 cells by modulating intracellular calcium levels [Ca^{+2}]i [16,17,3]. Thus, an investigation of kisspeptin's effects on [Ca^{+2}]i levels. Some studies showed that kisspeptin protects tissue damage as an antioxidant [3]. But no studies show the effect of kisspeptin on I/R injured ovary and uterus tissue. In our study SOD, GSH, MDA levels were increased in kisspeptin I/R and Kisspeptin + Oxytocin I/R group. Furthermore, moderate histopathologic damage was observed in kisspeptin injected groups.

Previous studies reported that the Oxytocin treated rats demonstrated almost normal structures of urothelium [1] and skeletal muscle cell [18] and ovarian tissue after ischemia-reperfusion [11]. Similarly, Alizadeh et al. showed that administration of Oxytocin in an experimental model of cardiac I/R injury in rats caused normalization of the ultrastructure of the cardiomyocytes. In the cardiovascular system, Oxytocin receptors are associated with the atrial natriuretic peptide-cGMP and nitric oxide-cGMP pathways, which reduce the force and rate of contraction and increase vasodilatation. Furthermore, it regulates tissue regeneration, stem cell differentiation, hypertrophy and glucose uptake potentiation, negative inotropic and chronotropic effects, anti-inflammatory and pro-inflammatory cytokines balance. Furthermore, Oxytocin activates mitoKATP and inhibits mPTP, by this way, it may block both apoptotic and cell-death pathways related to I/R [9].

Systemic Oxytocin administration has significant outcomes on blood pressure, vascular tone and cardiovascular and anti-inflammatory regulation. Moreover, its anti-inflammatory effects prevent epithelial damage and inflammatory cell infiltration in colon and uterus [19,20]. Oxytocin may have protective mechanism by this way on ovarian and uterine tissue.

In our study, we found that oxytocin protects uterine and ovarian tissue against I/R injury. Histopathologic damage was reduced in OXY + I/R and OXY + KISS + I/R groups compared to non-applied I/R group. Furthermore, biochemical findings of MDH, GSH levels and SOD activity were in parallel with histopathologic results. Protective effect of oxytocin and Kisspeptin-10 application together is stronger than oxytocin or Kisspeptin-10 alone application.

Consequently, our results suggest that Oxytocin and Kisspeptin as endocrine hormones can have antioxidant effects by exogenous application. These findings showed that some endocrine female hormones have potential for recovery of reproductive system by triggering antioxidant pathway. However, to understand the effects of Oxytocin and Kisspeptin hormones on antioxidant pathway we need further experimental investigations.

Conflicts of interest

The authors declare that they have no conflict of interest to disclose.

References

[1] Erkanli Senturk G, Erkanli K, Aydin U, Yucel D, Isiksacan N, Ercan F. The protective effect of oxytocin on ischemia/reperfusion injury in rat urinary bladder. Peptides 2013;40:82–8.
[2] Aydin M, Oktar S, Yonden Z, Ozturk OH, Yilmaz B. Direct and indirect effects of kisspeptin on liver oxidant and antioxidant systems in young male rats. Cell Biochem Funct 2010;28:293–9.
[3] Akkaya H, Kilic E, Eyuboglu Dinc S, Yilmaz B. Postacute effects of Kisspeptin-10 on neuronal injury induced by L-methionine in rats. J Biochem Mol Toxicol 2014;28(8):373–7.
[4] Bulbuler N, Sapmaz E, Akpolat N, Ustundag B, Kırkıl C. Effect of a bioresorbable membrane on postoperative adhesions and wound healing. J Rep Med 2003 Jul;48(7):547–50.
[5] Ellman GL, Courtney KD, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharm 1961;7:88–95.
[6] Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clin Chem 1988;34:497–500.
[7] Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the folin-phenol reagent. J Bio Chem 1951;193. 265–257.
[8] Ergenoglu M, Erbas O, Akdemir A, Yeniel AO, Yildirim N, Otulu F, et al. Attenuation of ischemia/reperfusion-induced ovarian damage in rats: does edaravone offer protection? Eur Surg Res 2013;51:21–2.
[9] Alizadeh AM, Faghihi M, Khori V, Sohonaki H, Pourkhalili K, Mohammadghasemi F, et al. Oxytocin protects cardiomyocytes from apoptosis induced by ischemia-reperfusion in rat heart: role of mitochondrial ATP-dependent potassium channel and permeability transition pore. Peptides 2012;36(1):71–7.
[10] Alizadeh AM, Mirzabeglo P. Is oxytocin a therapeutic factor for ischemic heart disease? Peptides 2013;45:66–72.
[11] Akdemir A, Erbas O, Gode F, Ergenoglu M, Yeniel O, Oltulu F, et al. Protective effect of oxytocin on ovarian ischemia-reperfusion injury in rats. Peptides 2014;55:126–30.
[12] Carden DL, Granger DN. Pathophysiology of ischemia-reperfusion injury. J Pathol 2000;190:255–66.
[13] Wall J. Antioxidants in prevention of reperfusion damage of vascular endothelium. TSMJ 2000;1:67.
[14] Abali R, Tasdemir N, Yuksel MA, Guzel S, Oznur M, Nalbantoglu B, et al. Protective effect of infliximab on ischemia/reperfusion injury in a rat ovary model: biochemical and histopathologic evaluation. Eur J Obstet Gynecol Reprod Biol 2013;171(2):353–7.
[15] Gennard E, Kelvin JAD. Calcium and oxidative stress: from cell signaling to cell death. Mol Biol 2001;38:713–21.

[16] Novaira HJ, Ng Y, Wolfe A, Radovick S. Kisspeptin increases GnRH mRNA expression and secretion in GnRH secreting neuronal cell lines. Mol Cell Endocrinol 2009;311(1–2):126–34.
[17] Ozcan M, Alcin E, Ayar A, Yilmaz B, Sandal S, Kelestimur H. Kisspeptin-10 elicits triphasic cytosolic calcium responses in immortalized GT1–7 GnRH neurons. Neurosci Lett 2011;492(1):55–8.
[18] Erkanli K, Erkanli Senturk G, Aydin U, Arbak S, Ercan F, Tuncdemir M. Oxytocin protects rat skeletal muscle against ischemia/reperfusion injury. Ann Vasc Surg 2013;27(5):662–70.
[19] Iseri SO, Sener G, Saglam B, Gedik N, Ercan F, Yegen BC. Oxytocin protects against sepsis-induced multiple organ damage: role of neutrophils. J Surg Res 2005 1;126(1):73–81.
[20] Moosmann B, Behl C. Secretory peptide hormones are biochemical antioxidants: structure-activity relationship. Mol Pharmacol 2002;61(2):260–8.
[21] Placer ZA, Cushman LL, Johnson BC. Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. Anal Biochem 1966;1:359–64.